REMARKS

Claims 12-23 are currently pending in this application. Claims 12 and 22 have been amended.

OBJECTIONS TO THE SPECIFICATION

Applicants have introduced section headings into the specification as requested by the examiner. The parenthetical structure has been removed as requested. The R group indicator has been inserted in Formula (II)3. The abbreviations in table I of page 10 designate the particular lipases as identified by their respective producers. These trade names are now introduced more completely.

OBJECTIONS TO THE CLAIMS

Claims 12 and 22 have been amended as requested by the examiner.

REJECTION UNDER 35 USC §112, ¶2

In claims 12 and 22, and also in the pertinent supporting paragraphs of the specification, applicants have replaced the language "altering the substrate specificity of" with the language "generating new catalytic activity in," the language "altered substrate specificity of" with "newly generated catalytic activity in," and "an alteration in the substrate specificity" with "newly generated catalytic activity." Further, in the pertinent portion of the specification, the definition of k_{cat} has been changed from "catalytic activity" to "rate of conversion."

As the examiner indicates, the present examples show random mutagenesis of

an enzyme unable to catalyze a particular reaction, and screening of the resulting mutant enzymes for activity in catalyzing the reaction. In several places in the specification as originally filed, applicants equated this generation of new enzymatic activities with alteration of the substrate specificity of the enzyme. In describing the contemplated alteration of substrate specificity, applicants described it as being a function of the affinity of the enzyme for the substrate and the catalytic activity of the enzyme on that substrate. These two factors were represented by K_M and k_{cat}, respectively.

Enzymic activity, according to the Oxford Dictionary of Biochemistry and Molecular Biology, is "the rate of reaction of substrate that may be attributed to catalysis by an enzyme" (p.210, see attached excerpt). It is "now obsolete" and has been superseded by the term "catalytic activity" (*id.*). Catalytic activity of an enzyme, in turn, is defined as "the property measured by the increase in the rate of conversion of a specified chemical reaction that the enzyme produces in a specified assay system. ... [I]t is ... conceptually different from rate of conversion although measured by and equidimensional with it" (*id.*, p.97).

Applicants have described their invention through the specification in terms of substrate/enzyme binding (K_M) and rate of conversion (k_{cat}). As indicated in the Oxford Dictionary entry cited above, catalytic activity is the presently accepted term of art denoting the combined effect of these factors. Applicants respectfully submit that no new matter has been introduced by the above-indicated amendments.

Additionally, in claims 12 and 22, applicants have introduced additional structural

language to further define the contemplated functional derivatives. This language is found in the specification at p.5:41-43. A functional derivative of Escherichia coli XL-1 Red is one which carries the genetic markers relA1, mutS, mutT and mutD5. The specification further indicates that these markers "result in a distinctly increased mutation rate in the organisms" (p.5:43-45). Applicants respectfully submit that with this additional structural information, the metes and bounds of the retained function are clearly defined.

Finally, in claim 20, the reference to lipase PS and lipase AH should be clear in light of the amendment to Table I on page 10 of the specification.

REJECTION UNDER 35 USC §112, ¶1

WRITTEN DESCRIPTION

Originally filed claims are subject to a strong presumption that there exists in the specification sufficient written description to support them. The present claims are identical to those originally filed in recitation a functional derivative of *E. coli* strain XL1-Red. The present specification, also as originally filed, states that "[a] functional derivative of this strain [*E. coli* XL1-Red] preferably means *Escherichia coli* strains which contain the following genetic markers: relA1, mutS, mutT and mutD5" (p.5:41-43). Further, the specification indicates that "[t]hese genetic markers result in a distinctly increased mutation rate in the organisms" (p.5:43-45).

In claiming the method of the present invention, applicants originally recited use of "the *Escherichia coli* strain XL1-Red or ... a functional derivative" (original claim 1).

This language was amended to introduce the word "thereof" following "a functional derivative" to further clarify the nature of the derivative as being derived from *E. coli* XL1-Red. Applicants submit that the specification teaching cited above clearly supports this range of functional equivalents as originally claimed.

Applicants have amended claims 12 and 22 to include the recited genetic markers a structural requirement for the functional derivatives of *E. coli* XL1-Red used in the invention. This amendment is not seen to reduce the originally contemplated scope of the invention. It does make more clear the range of functional equivalents presently claimed. As indicated by the CCPA,

> we are of the opinion that the PTO has the initial burden of presenting evidence or reasons why persons skilled in the art would not recognize in the disclosure a description of the invention defined by the claims.

(*In re Wertheim*, 541 F.2d 257, 263, 191 USPQ 90, 97 (CCPA 1976).) Applicants respectfully request the examiner to provide such evidence or reasons in light of the above discussion.

The examiner applies *Regents of the University of California v. Eli Lilly* in her written description analysis of the enzyme aspect of the present invention (43 USPQ2d 1398 (Fed. Cir. 1997), *cert. denied*, 523 U.S. 1089 (1998)). In *Regents*, the University of California (UC), in pertinent part, asserted infringement of claims directed to mammalian and human cDNA, and to recombinant plasmids and microorganisms containing sequences coding for vertebrate insulin mRNA (*id.*). As the examiner indicates, the CAFC cited precedent and held that support for such claims had to be shown in the form of actual chemical structures, and not through prophetic examples

and instructions for obtaining the claimed cDNA (*id.*).

The examiner miscites this case, however, and in a key place. In the examiner's citation, she quotes the CAFC as stating that

> "... In claims to *methods using* genetic material, however, a generic statement such as 'vertebrate insulin cDNA' or 'mammalian insulin cDNA,' without more, is not an adequate written description of the genus because it does not distinguish the genus from others, except by function. ..." in UC v. Lilly at *24-*25.

(office action of December 10, 2002, p.8, emphasis supplied.) The italicized words are not found in the text as reported in the United States Patent Quarterly, Second Series. Inclusion of these words shifts the applicability of *Regents* from structural claims exclusively to one encompassing method claims, as well.

The actual language from *Regents* reads

> In claims to *genetic material*, however, a generic statement such as "vertebrate insulin cDNA" or "mammalian insulin cDNA," without more, is not an adequate written description of the genus because it does not distinguish the claimed genus from others, except by function.

(43 USPQ2d at 1406, emphasis supplied.) As none of the present claims are directed to genetic material, *per se*, *Regents* is inapplicable to the present written description analysis.

Applicants submit that there exists adequate written description in the specification to support the claimed subject matter of claims 12-23, and respectfully request that the rejection under 35 USC §112, ¶1 for lack of adequate written description be withdrawn.

ENABLEMENT

As indicated by the examiner, "[e]nablement is not precluded by the necessity for some experimentation such as routine screening" (*In re Wands* 8 USPQ 2d 1400, 1404 (Fed. Cir. 1988)). The test for determining "what constitutes undue experimentation in a given case requires the application of a standard of reasonableness, having due regard for the nature of the invention and the state of the art" (*Id.*, citing *Ansul Co. v. Uniroyal, Inc.* 169 USPQ 759, 762-63 (2d Cir. 1971), *cert. denied*, 404 U.S. 1018, 172 USPQ 257 (1972)). Further,

> The test is not merely quantitative, since a *considerable amount* of experimentation is permissible, *if it is merely routine*, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed ... .

(*Id.*, citing *In re Jackson*, 217 USPQ 804, 807 (BPAI 1982), emphasis supplied.)

As a guide in applying this reasonableness standard, certain factors have been adopted. These include

> (1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claims.

(*Id.*, citing *Ex parte Forman,* 230 USPQ 546, 547 (BPAI 1986).) As the Federal Circuit held in *In re Wands*, the present invention does not require *undue* experimentation, even though the *quantity* of experimentation necessary to practice the invention is high (*Id.* at 1406-1407).

In considering the quantity of experimentation necessary to successfully practice the presently claimed invention, the examiner observes that

> The amount of experimentation to randomly screen for a "novel" enzyme activity is wholly dependent on the type of substrate looking to be used by the "new" enzyme - a substrate very similar to the original is likely to take little experimentation while a substrate unlike the original is unlikely to produce any positive result at all.

(office action, p.10.) Whether this assertion is true or not, the process of mutagenesis and screening claimed is composed of several different procedures which are routine in the art. The quantity of experimentation may vary depending on the particular mutagenesis undertaken, and yet, "a *considerable amount* of experimentation is permissible, *if it is merely routine*" (*In re Wands*, at 1404 citing *In re Jackson* at 807, emphasis supplied).

The present claims require the following procedures: isolating a gene of interest, transforming *Escherichia coli* strain XL1-Red with this gene, incubating the transformed *E. coli* XL1-Red, retrieving the gene from the subsequent generations of *E. coli* XL1-Red, transforming a second microorganism with the retrieved gene, incubating the transformed microorganism, and screening for the desired enzymatic activity. Each of these procedures is, and was at the time of filing, routine. The possibility that a protein with no known function, or no known structure, could be employed in this process with the result that the many iterations and screenings give no positive results does not change the fact that all of the procedures are "*merely routine*" (*Id.*).

No indication has been given as to the pertinence of quantity in determining undue experimentation, where the procedures are, in fact routine. There is no support

in the case law discussion for a cut-off point at which the quantity of routine experimentation becomes undue. As the Federal Circuit framed the issue in *In re Wands*,

> The nature of monoclonal antibody technology is that it involves screening hybridomas to determine which ones secrete antibody with desired characteristics. Practitioners of this art are prepared to screen negative hybridomas in order to find one that makes the desired antibody. No evidence was presented by either party on how many hybridomas would be viewed by those in the art as requiring undue experimentation to screen. ...

(*Id.* at 1406.)

As to the amount of direction or guidance presented, the procedures required to practice the invention are routine and well-known in the art, and "a patent need not teach, and preferably omits, what is well known in the art" (*Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1384, 231 USPQ 81, 94 (Fed. Cir. 1986), citing *Lindemann Maschinenfabrik v. American Hoist and Derrick*, 730 F.2d 1452, 1463, 221 USPQ 481 489 (Fed. Cir. 1984)). Again, considerations such as of which enzyme gene to employ or which substrate to target are beside the point of enablement of claims involving routine procedures.

Applicants have provided working examples of how the present procedures are carried out, and the routine nature of these procedures, i.e., the state of the art and the relative skill of those in the art, should obviate any perceived necessity for additional working examples. As to the predictability of the art, applicants again point out that the claimed procedures are routine, and that those of skill in the art understand that the quantity of this routine experimentation will vary depending on the similarities between

the enzyme's natural substrate and the targeted substrate. This quantitative factor, however, is not decisive in light of the routine nature of the required experimentation.

Applicants submit that claims 12-23 are enabled by the present specification, and request that the rejection under 35 USC §112, ¶1 for lack of enablement be withdrawn.

REJECTION UNDER 35 USC §103(A)

Claims 12 and 16 are drawn to use of *ether* hydrolases specifically, and not to use of *hydrolases* generally. Ether hydrolases are classified as EC 3.3.2, whereas alkaline phosphatases, such as those of Greener, et al., are classified as EC 3.1.3.1, and are phosphoric mono*ester* hydrolases, EC 3.1.3. The examiner has not met her burden to show in the prior art a teaching or suggestion of each claim element, some suggestion or motivation to make the claimed invention, and a reasonable expectation for success in doing so (*see, e.g., In re Fine*, 837 F.2d 1071, 5 USPQ2d 1596 (Fed. Cir. 1988); *In re Jones*, 958 F.2d 347, 21 USPQ2d 1941 (Fed. Cir. 1992); *In re Merck & Co., Inc.*, 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986); *In re Royka*, 490 F.2d 981, 180 USPQ 580 (CCPA 1974)). Applicants request that the rejection of claims 12, 16 and 21 as obvious over Wilks et al., in view of Greener et al., under 35 USC §103(a), be withdrawn.

CONCLUSION

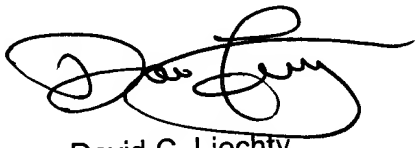
In view of the foregoing amendments and remarks, applicants consider that the

rejections of record have been obviated and respectfully solicit passage of the application to issue.

Please find attached a check for $110.00 for a one month extension of time fee.

Please charge any shortage in fees due in connection with the filing of this paper, including Extension of Time fees to Deposit Account No. 11-0345. Please credit any excess fees to such deposit account.

Respectfully submitted,
KEIL & WEINKAUF



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VERSION WITH MARKINGS TO SHOW CHANGES MADE IN THE SPECIFICATION

Please amend the title as follows:

The generation of new catalytic activities in ~~alteration of the substrate specificity of~~ enzymes

Please enter the following heading on page 1, line 6 of the specification.

I BACKGROUND

Please amend the paragraph on p.1:5-6 as follows:

The invention relates to a method for generating new catalytic activities ~~altering the substrate specificity of enzymes~~.

Please amend the paragraphs on p.2:44-p.3:17 as follows:

The disadvantage of all the mutagenesis methods mentioned is that it is possible to optimize only enzymatic activities which are present. If new enzymatic reactions~~, i.e., new substrate specificities of the enzymes,~~ are required, for example for cleaving a new substrate, it is necessary first to search for this new enzymatic activity in an elaborate screening of natural forms. As a rule, it is then necessary to optimize the enzyme further.

I2

It would therefore be desirable to have a method which makes it possible to generate new enzymatic activities~~, i.e., is able to alter the substrate specificity of enzymes~~.

SUMMARY

It is an object of the present invention to develop a novel widely applicable method which does not have the abovementioned disadvantages and which is able to generate new catalytic activities in ~~alter the substrate specificity of~~ enzymes rapidly and straightforwardly.

I2 cont

We have found that this object is achieved by a method for generating new enzymatic activities in ~~altering the substrate specificity of~~ enzymes, which comprises carrying out the following steps:

Please amend the paragraphs on p.3:17-4:27 to read as follows:

d) incubating this microorganism to detect the enzyme activity on or in at least one selection medium which comprises at least one enzyme substrate which makes it possible to recognize unaltered catalytic activity in ~~substrate specificity of~~ the enzyme, with or without other indicator substances,

I3

e) selecting the microorganisms which show the new enzymatic activity ~~an alteration in the substrate specificity~~.

DETAILED DESCRIPTION

Generation of new catalytic activities ~~Alteration of the substrate specificity~~ in the novel method means that the enzymes having been subjected to the method are able to convert substrates which they were previously unable to convert, because the affinity of the enzyme for the substrate was too low (i.e., = high K_M) and/or the rate of conversion ~~catalytic activity~~ (= k_{cat}) of the enzymes was too low. In these cases, the ratio k_{cat}/K_M is zero or almost zero, i.e., catalysis does not occur. The generation of a new catalytic activity ~~alteration in the substrate specificity~~ reduces the K_M or increases the k_{cat}, or both~~, i.e., the ratio k_{cat}/K_M becomes greater than zero~~. A catalytic reaction occurs. The enzyme converts the new substrate after the mutagenesis.

I³ Cont

It is possible in principle for new catalytic activities to be generated in all enzymes ~~the substrate specificity of all enzymes to be altered~~, and preferably new catalytic activities are generated in ~~the substrate specificity of~~ hydrolases ~~is altered~~ in the novel method. Hydrolases for the 3rd class of enzyme (= 3..) in the IUB nomenclature system. Hydrolases are preferred in the novel method because, as a rule, a simple detection reaction for them exists and, in many cases, they are used in industrial syntheses. It is particularly preferred to generate new enzymatic activities in ~~alter the substrate specificity of~~ hydrolases selected from the group consisting of proteases, lipases, phospholipases, esterases, phosphatases, amidases, nitrilases, ether hydrolases, peroxidases and glycosidases, very particularly preferably lipases, esterases, nitrilases or phytases.

After the new catalytic activity ~~substrate specificity~~ has been generated ~~altered~~ in the method according to the invention, the enzyme reaction can take place without or with selectivity in the case of chiral starting compounds, i.e., the reaction results in racemic or optically active products. Preferred new activities ~~alterations~~ result in selective catalytic activity ~~alterations in the substrate specificity~~ such as regio-, chemo- or stereoselective or in regio-, chemo- and/or stereoselective reactions.

I3

Please amend the paragraph on p.5:31 to 6:2 as follows:

Particularly suitable for the methods according to the invention for producing mutations (step b, Figure 1) is the Escherichia coli strain XL1 Red (= Epicurian coli XL-1 Red), which is marketed by Stratagene La Jolla, CA. It carries the following genetic markers: Δ[mcrA]183, Δ[mcrCB-hsdSMR-mrr]173, endA1, supE44, thi-1, gyrA96, relA1, mutS, mutT, mutD5, lac. MutS is a mutation in the mismatch repair pathway, mutT is a mutation in the oxo-dGTP repair pathway and mutD5 is a mutation in the 3'-5' exonuclease subunit of DNA polymerase III. Competent cells of this strain can be purchased from Stratagene under order number 200129. A functional derivative of this strain preferably means Escherichia coli strains which contain the following genetic markers: relA1, mutS, mutT and mutD5. These genetic markers result in a distinctly increased mutation rate in the organisms. They should therefore not be incubated on agar plates or in a culture medium for too long, because otherwise they lose their vitality.

I4

Please amend the paragraphs on p.6:4-p.7:34 as follows:

For detection of the newly generated catalytic activity, ~~altered substrate specificity (= mutations in the enzyme used)~~ it is possible and advantageous, in the case where vectors have been used, for the DNA initially to be isolated from the E. coli strain XL1 Red or its functional derivative and be inserted into a microorganism which has no corresponding enzyme activity (step c, Figure 1). If, for example, an esterase is introduced into these selection organisms, these microorganisms must not have any esterase activity which cleaves the ester used for selection for the newly generated catalytic activity in ~~an alteration in the substrate specificity of~~ the esterase. Other esterase activities in this organism do not interfere with the selection. ... It is also possible for the DNA of the microorganisms which show the newly generated catalytic activity ~~an altered substrate specificity~~ after selection to be introduced without isolation by conjugation or using phages or viruses or by transformation into the strain E. coli XL1 Red or a functional derivative for a further selection cycle (= see Figure 1, dotted line). It is possible in this way for the method according to the invention to be performed one or more times in sequence. The DNA is in this case transferred from the E. coli strain XL1 Red or its functional derivatives to the selection organisms and finally returns to the E. coli strains for a new selection cycle.

I5

Selection microorganisms which are suitable in principle in the method according to the invention are all prokaryotic or eukaryotic microorganisms, although they must have no enzymatic activity which could impede the selection. This means either that the

microorganisms have no enzymatic activity which is sought, i.e., the substrate(s) used for selecting the newly generated catalytic activity ~~altered substrate specificity~~ are not converted by the enzymes in the selection microorganism, or that only a small enzymatic activity of this type is present in the microorganisms and still permits selection. Suitable and advantageous microorganisms for the method according to the invention are Gram-positive or Gram-negative bacteria, fungi or yeasts. Those preferably used are Gram-positive bacteria such as Bacillus, Rhodococddus, Streptomyces or Nocardia, or Gram-negative bacteria such as Salmonella, Pseudomonas or Escherichia. Escherichia coli strains are very particularly preferably used. The genus and species or the membership of a family or kingdom of the microorganisms used for the selection is of minor importance as long as it allows selection of the newly generated catalytic activity ~~altered substrate specificity~~.

I5 Cont

To select for newly generated catalytic activity ~~altered substrate specificity~~, the microorganisms are incubated, for detection of the enzyme activity, on or in at least one enzyme substrate which makes it possible to recognize the newly generated catalytic activity in ~~an altered substrate specificity of~~ the enzyme (Figure 1, step d). this selection medium may contain other indicator substances which improve recognition of the desired newly generated enzymatic activity ~~alteration~~. Possible additional indicator substances of this type are, for example, pH indicators.

Figure 1 depicts the individual steps in the method taking the example of the use of a

vector (1). Step a shows the introduction of the DNA (2) via the vector into the strain Escherichia coli XL1 Red or into a functional derivative (3) of this strain. The DNA of the enzyme is mutated in these organisms [asterisks in Figure 1 indicate diagrammatically by way of example the mutations in the DNA (2)]. The mutated vectors (4) are then reisolated from the strain Escherichia coli XL1 Red or its functional derivatives and subsequently transformed, directly or after storage, into the selection organisms (5) (step c).. These organisms are finally plated out on at least one selection medium (6) and thus the newly generated catalytic activity ~~altered enzyme substrate specificity~~ is identified by, for example, a growth assay and/or a visual assay (step d). Positive clones which show the newly generated catalytic activity ~~an altered substrate specificity~~ are finally selected, and the mutated gene coding for the altered enzyme can be isolated (step e). The method can be repeated several times using the mutated gene [Figure 1, dotted line (7)].

I5

Please amend the paragraph on p.8:39-44 as follows:

The mixing ratio of said nutrients depends on the mode of incubation (= fermentation) and is established in the individual case. The medium components may all be present at the start of the fermentation after they have been sterilized, if necessary, separately or together, or else can be added as required during the incubation.

I6

Amend the paragraphs on p.9:7-45 to read as follows:

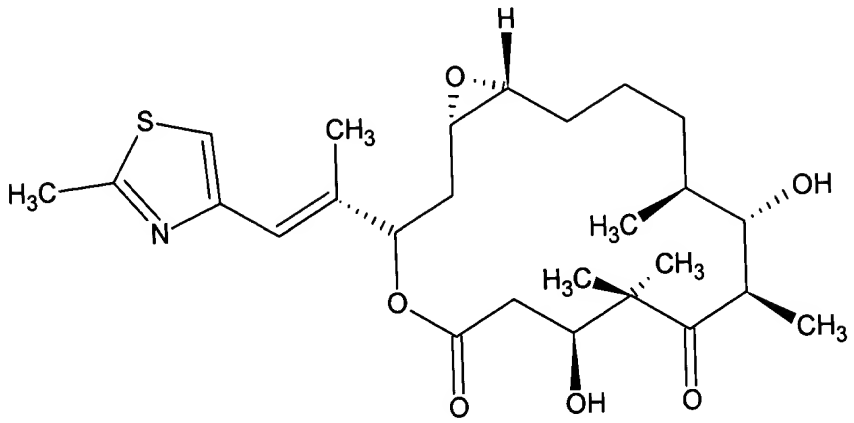
The culturing conditions are established so that the organisms (i.e., = Escherichia coli strain XL1 Red and selection organisms) grow optimally and that the best possible yields are obtained. Culturing is preferably carried out at from 15°C to 40°C, particularly advantageously from 25°C to 37°C. The pH is preferably kept in the range from 3 to 9, particularly advantageously from 5 to 8. In general, the incubation time of from 1 to 240 hours, preferably from 5 to 170 hours, particularly preferably from 10 to 120 hours, is sufficient, but longer incubation times may also be necessary in a few cases for the mutagenesis or detection.

I7

The newly generated catalytic activity ~~altered substrate specificity~~ can, after identification of the corresponding clones, advantageously be checked again in an in vitro assay.

Examples:

Retrosynthetic analysis showed that the 3-hydroxy esters see formula II, (1) and (3) are favorable starting material for synthesizing the macrolide antibiotic epothilon A (see formula I).



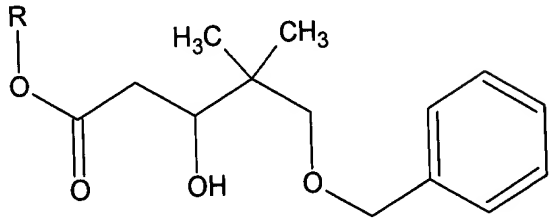
(I)

I7

1: R = CH_2CH_3

~~3: $CH_2CH(OH)CH_2OH$~~

3: R = $CH_2CH(OH)CH_2OH$



(II)

Please amend the table on page 10 as follows:

I8

Enzyme from	Manufacturer
Pseudomonas cepacia (= Lipase PS ®)	Amano, Nagoya, Japan
Pseudomonas cepacia (= Lipase AH ®)	Amano, Nagoya, Japan
Acylase "Amano" ® (ACS)	Amano, Nagoya, Japan
Rhizopus delamar (Lipase D ®)	Amano, Nagoya, Japan
Rhizopus javanicus (Lipase F-AP15 ®)	Amano, Nagoya, Japan
Candida rugosa (Lipase AY ®)	Amano, Nagoya, Japan
Mucor javanicus (Lipase M ®)	Amano, Nagoya, Japan
Penicillium roquefortii (Lipase R ®)	Amano, Nagoya, Japan
Penicillium cyclopium (Lipase G50 ®)	Amano, Nagoya, Japan

Chromobacterium viscosum (crude)	Toyo Jozo, Tokyo, Japan
Chromobacterium viscosum (pure)	Toyo Jozo, Tokyo, Japan
Rhizomucor miehei	Biocatalysts Ltd. Pontypridd, UK
Humicola lanuginosa	Biocatalysts Ltd. Pontypridd, UK
Rhizomucor miehei (Lipozyme, immobilized ~~immob.~~)	Novo, Bagsvaerd, Denmark
Candida antarctica lipase B (Novozyme SP435®, immobilized ~~immob.~~)	Novo, Bagsvaerd, Denmark
Candida antarctica lipase B (Novozyme SP525®, free)	Novo, Bagsvaerd, Denmark
Candida antarctica lipase A (Novozyme SP526®, free)	Novo, Bagsvaerd, Denmark
Candida antarctica lipases A, B (Novozyme SP382®, free)	Novo, Bagsvaerd, Denmark
Pig liver esterase	Fluka, Buchs, Switzerland
Esterase from Thermoanaerobium brockii	Fluka, Buchs, Switzerland

I8

Please amend the paragraph on p.11:7-8 as follows:

I9

The esterase was mutagenized using the strain Escherichia coli XL-1 Red (= Epicurian coli XL-1 Red).

MARKED UP VERSION OF AMENDMENTS IN THE CLAIMS

Please amend claims 12 and 22 as follows:

12. (currently amended) A method for generating new catalytic activity in ~~altering the substrate specificity of~~ an enzyme, comprising the steps of:

a) introducing a DNA sequence coding for the enzyme into the *Escherichia coli* strain XL1-Red or into a functional derivative thereof which carries the genetic markers relA1, mutS, mutT and mutD5,

b) incubating the transformed *Escherichia coli* strain XL1-Red or its functional derivative to generate mutations in the DNA sequence,

c) transferring the mutated DNA sequence from the transformed *Escherichia coli* strain XL1-Red or its functional derivative to a microorganism which has no impeding enzyme activity,

I 10

d) incubating this microorganism to detect the enzyme activity in at least one selection medium which comprises at least one enzyme substrate to recognize newly generated catalytic activity in ~~altered substrate specificity of~~ the enzyme, with or without other indicator substances, and

e) selecting the microorganisms which show newly generated catalytic activity ~~an alteration in the substrate specificity~~, said microorganisms in steps c) ~~b)~~, d) and e) being a member selected from the group consisting of bacteria, fungi and yeasts,

wherein the enzyme is selected from the group consisting of lipases, amidases, nitrilases, ether hydrolases, peroxidases, glycosidases and phytases.

22. (currently amended) A method for generating new catalytic activity in ~~altering the substrate specificity of~~ an enzyme, comprising the steps of:

a) introducing a DNA sequence coding for the enzyme into the *Escherichia coli* strain XL1-Red or into a functional derivative thereof which carries the genetic markers relA1, mutS, mutT and mutD5,

b) incubating the transformed *Escherichia coli* strain XL1-Red or its functional derivative to generate mutations in the DNA sequence,

c) transferring the mutated DNA sequence from the transformed *Escherichia coli* strain XL1-Red or its functional derivative to a microorganism which has no impeding enzyme activity,

d) incubating this microorganism to detect the enzyme activity in at least one selection medium which comprises at least one enzyme substrate to recognize newly generated catalytic activity in ~~altered substrate specificity of~~ the enzyme, with or without other indicator substances, and

e) selecting the microorganisms which show newly generated catalytic activity ~~an alteration in the substrate specificity~~, said microorganisms in steps c) ~~b)~~, d) and e) being a member selected from the group consisting of bacteria, fungi and yeasts,

wherein the enzyme is an esterase selected from the group consisting of *Pseudomonas fluorescens* esterase, pig liver esterase and *Thermoanaerobium brockii* esterase.